

Effect of Cinnamon Extract and Chlorhexidine Gluconate (0.2%) on the Clinical Level of Dental Plaque and Gingival Health: A 4-Week, Triple-Blind Randomized Controlled Trial

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Abstract

Aim: To compare the effect of cinnamon extract, chlorhexidine mouthwash and placebo on dental plaque level and gingivitis.

Materials and methods: One hundred five healthy dental and medical students aged 21 to 25 years participated in the study. The subjects were randomly divided into three groups: i.e., the cinnamon group, the chlorhexidine gluconate mouthwash group and the placebo (distilled water) group. Data were collected at baseline, the 15th and the 30th day. Plaque was disclosed using erythrosine disclosing agent and scores were recorded using the Quigley and Hein plaque index modified by Turesky-Gilmore-Glickman. Gingival scoring was done by the gingival index of Löe and Silness. Statistical analysis was carried out to compare the effect of all three treatments groups; $p \leq 0.05$ was considered statistically significant.

Results: The chlorhexidine group showed the maximum decrease in both plaque and gingival scores, followed by cinnamon extract, but the result was statistically insignificant. The plaque and gingival scores remained almost unchanged in the distilled water group.

Conclusion: The results of the present study indicate that cinnamon may prove to be an effective agent owing to its ability to reduce plaque level and gingivitis.

Key words: *cinnamon extract, chlorhexidine, plaque index, gingival index.*

Introduction

Natural products have a long history of treating oral diseases (Dhinahar and Lakshmi, 2011). Oral deposits such as plaque and calculus have a great impact on oral health-related quality of life and may lead to systemic and threatening diseases. These oral deposits may lead to the accumulation of microorganisms that have a strong relationship with oral diseases (Rishton, 2008). Plaque-induced gingivitis is the most frequent periodontal disease. It affects around 90% of the population worldwide (Barnett, 2006). Thus, removal of plaque and prevention of gingivitis is the mainstay in the prevention of periodontal diseases. There are several mechanical anti-plaque agents

available that reduce plaque effectively. Some adjuncts, such as mouthrinses, are used as oral hygiene products that deliver active agents to the oral structures. These adjuncts act by inhibiting bacterial colonization, growth, and metabolism (Paraskevas, 2005). Alternative therapies are needed to treat these oral diseases because of increased bacterial resistance to regular products (Gupta *et al.*, 2014a; Gupta *et al.*, 2014b). Apart from the conventional health system, alternative medicines have also been used since time immemorial for treatment of various ailments (Gupta *et al.*, 2014c; Dhinahar and Lakshmi, 2011; Gupta *et al.*, 2014d).

The most efficacious and safe agent used to control plaque is chlorhexidine. It is regarded as the benchmark control in plaque removal, though it has some side effects such as altered taste sensation and staining of the teeth and the tongue, which limits its use. Some oral bacteria develop resistance to the antibacterial activity of chlorhexidine (Arunachalam *et al.*, 2012).

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These limitations of chlorhexidine led to the development of naturally occurring oral hygiene products. Cinnamon extract may act as a good and cost-effective oral hygiene product.

Today, a large proportion of the population prefers natural products (Ceylani and Daniel, 2004; Gupta *et al.*, 2014e). These products have been shown to be a good alternative to synthetic chemical substances for periodontal disease prevention (Gupta *et al.*, 2014e). Cinnamon (*Cinnamomum zeylanicum*) is a member of the Lauraceae family. It is one of the main herbs to be used extensively for treatment of several conditions. Cinnamon is used in dried form or ground form. The bark of the cinnamon tree contains an essential oil called cinnamonaldehyde, which give cinnamon its characteristic flavor and aroma. The inner bark of the cinnamon tree has been used as a spice for thousands of years. Cinnamon is thought to have many health benefits, so it is used as an herbal medicine. From historical time, cinnamon has been used as a medicine for colds, flatulence, nausea and diarrhea by improving energy, vitality, and circulation (Rashad, 2008; Ooi *et al.*, 2006). Cinnamon also inhibits the growth of food-borne bacteria such as *Salmonella enterica* and *Escherichia coli* (Arora and Kaur, 1999).

Many studies have found that cinnamon has antibacterial, anti-inflammatory and antifungal properties (Quale, 1996; Burt, 2004). However, no study has been conducted to evaluate the antiplaque and anti-gingivitis properties of cinnamon extract. Because of its antimicrobial and anti-inflammatory activity, it was hypothesized that rinsing with cinnamon-containing mouthwash could probably lead to a reduction in dental plaque and gingival inflammation. Thus, the aim of the present study was to determine the effect of an herbal mouthwash containing the extract of cinnamon on periodontal parameters such as gingivitis and dental plaque in subjects after rinsing for 15 days and 30 days. Additionally, we compared it with chlorhexidine and placebo.

Materials and methods

This triple-blind, randomized controlled trial, a three-group parallel study, was conducted in the Department of Public Health Dentistry on male and female university student volunteers from the Teerthanker Mahaveer Dental College and Research Centre. The protocol was approved by the Institutional Review Board (IRB) of Teerthanker Mahaveer University. All subjects signed an IRB-approved consent form. A pilot study was done on 10 patients in each group to determine the feasibility of the study; the results are not included in the present study.

Inclusion and exclusion criteria

Students who gave informed consent were included. Those who had not undergone any dental treatment, and who had not had any antibiotic or anti-inflammatory drug therapy

in the preceding 6 months were included in the study. Participants with a baseline DMFT (decayed/missing/filled teeth) score of 2 to 5 were included in the study.

Participants with any systemic diseases/conditions (Karim *et al.*, 2014; Gupta *et al.*, 2014), fibrotic gingival enlargement, smoking, or oral hygiene index score (OHIS; Greene and Vermillion, 1964) of 3.1 - 6.0 were excluded from the study. Those volunteers who had used antibiotics or mouthwash for five consecutive days or corticosteroids in the past 30 days or participants with sensitivity to any mouthwash were excluded from the study. Those subjects who were using any removable prostheses or orthodontic appliance were excluded from the study. Participants who had undergone oral prophylaxis to eliminate plaque and calculus in the past 15 days were excluded. Based on clinical examination, subjects having pockets (ADA type III and type IV) were excluded together with those who did not give consent for study.

This study employed a randomized, triple-blinded controlled intervention of 4 weeks duration. All undergraduate dental student volunteers from Teerthanker Mahaveer University were subjected to clinical examination, and a sampling frame ($n = 105$; 35 in each group) of those who fulfilled the inclusion and exclusion criteria was prepared. A total of 105 volunteers were randomly allocated into the three study groups through computer-generated random numbers. Random allocation of mouthrinses using the lottery method was done. The computerized generation of random allocation sequence and the allocation of subjects was done by another examiner who was not involved in the investigation.

The total sample size was 105 (35 subjects in each group). The sample size was calculated for α error fixed at $< 5\%$ ($p < 0.05$) and β fixed at 20%, expected mean difference 2.319 and standard deviation 2.581. Based on the above calculation, the minimum sample required in each group was 35 subjects. Students were allocated in three groups.

All three groups followed the same oral hygiene instructions, and differed only in allocated mouthrinses. All three solutions were made to be the same color and taste as much as possible with the help of the Department of Pharmacy, Teerthanker Mahaveer University, and kept in coded containers that were decoded later. All mouthrinses were packaged in opaque bottles and brown paper bags and no labelling was carried out, thus blinding the examiner and subject with respect to treatment arm. This was a triple-blind study in which coding was done for each group and individuals, wherein the investigator and study subjects as well as the statistician were not aware to which group the subjects belonged. Students in all the three groups were instructed to rinse their mouth with 10 ml of mouthwash twice daily after breakfast and again after lunch for one minute, and not to rinse with water thereafter, for 30 days. Twice daily rinsing was supervised throughout the week in the Department of Public Health Dentistry by a research assistant.

Unsupervised rinsing took place on the weekends in the hostel rooms in front of trained wardens (10) and was also documented in a diary completed by the subject. Students were instructed to continue with their daily oral hygiene measures and to refrain from the use of any antimicrobial agent other than the prescribed mouthrinse during the experimental period. The subjects were instructed to brush twice daily in horizontal scrub method and to rinse the toothbrushes under running tap water twice after brushing under the supervision of a trained hostel warden. All students were given a personal set containing the same type of new toothbrush and fluoride whitening toothpaste to decrease the possible side effects of staining and lessen examiner bias. Amount of toothpaste remaining in the toothpaste tube was checked weekly to confirm adherence of students to twice-daily brushing instruction. A single examiner, who was trained and calibrated to record the plaque and gingival scores, recorded the findings at all three intervals and for all three groups. The recorder was blinded to the type of the mouthwash used by participants. Calibration was done in multiple sessions by applying the indices on 10 selected subjects in the Department of Public Health Dentistry on the given dates. The faculty and examiner both applied the selected indices separately and data was fed into a spreadsheet. Inter-examiner reliability was calculated by applying the kappa statistic. The kappa coefficients were .80 and .86 respectively for plaque index and gingival index. The concordance, as reflected by kappa statistics, was high for all the selected indices. The examiner did a repeat oral examination and applied the same indices on the same individuals after a gap of one week, and data were subject to test - retest reliability. The correlation coefficient reflected a high degree of concordance: .82 and .86 for plaque and gingival index, respectively. Parameters such as gender, smoking status, level of education, monthly family income, number of teeth present, plaque index, gingival index and gingival bleeding index were not significantly different among the groups at baseline (all $p > 0.1$).

The data were collected at baseline, and at 15 and 30 days. The plaque was disclosed using erythrosine disclosing agent and scores were recorded using the Quigley and Hein plaque index as modified by Turesky-Gilmore-Glickman (Turesky *et al.*, 1970). The gingival score was recorded using the gingival index of Löe and Silness (1963).

Preparation of cinnamon extract

Fresh cinnamon bark was taken from the Botanical Garden of Teerthanker Mahaveer University and was authorized by the Botanical Department of the university in April 2013. It was ground to a fine powder in a mechanical grinder. Ten grams of finely powdered cinnamon were mixed with 100 ml of sterile deionized water and kept in a water bath in a round-bottomed flask at 55 - 60° C for five hours, then filtered through sterile filter paper (Whatman, UK). The aqueous extract was decanted, clarified by filtration through a muslin cloth and evaporated in a porcelain dish at 40° C, which resulted in the dried extract. This dried extract was suspended in polyethylene glycol (PEG) 400 (20% v:v) and sterile distilled water to give a final concentration of 20% w:v.

Statistical analysis

The data were analyzed using SPSS version 17. Analysis of variance (ANOVA) was used followed by post-hoc least squares difference test (LSD). A p value of 0.05 was taken to be significant.

Results

Plaque and gingivitis score

The descriptive baseline statistics of the three groups are depicted in Table 1. The intra-examiner error was within acceptable limits and the power of the study was found to be 0.971 using power and sample size program software. There were no reports of adverse reactions to any of the mouthrinses used. Analysis of variance (ANOVA) was used to analyze the reduction in plaque and gingivitis in the three groups. ANOVA (Table 2) was carried out to assess the intra- and inter-group variations for plaque and gingivitis, respectively. Significant decrease was noted in plaque and gingivitis in both the cinnamon and chlorhexidine groups at 15 days and 30 days ($p < 0.05$; Table 2). There was a progressive decline in the plaque and gingivitis scores at the 5% level of significance. The chlorhexidine group showed remarkably greater reduction as compared to the cinnamon group, but it was not statistically significant. Multiple comparisons were obtained by post-hoc LSD test. The difference in the decrease in plaque ($p = 0.609$ at 15 days and $p = 0.702$ at 30 days) and gingivitis ($p = 0.748$ for 15 days and $p = 0.817$ for 30 days) between the cinnamon group and the chlorhexidine group was not statistically significant.

Table 1. Demographic analysis of the study groups using different mouthrinses

	Cinnamon	Chlorhexidine	Placebo	p value*
Total number of participants	35	35	35	
Age (mean 23 years) (range 18 - 26 years)	23.17 \pm 2.45	22.72 \pm 2.08	23.13 \pm 2.39	0.79
Number of male/female subjects	17/18	16/19	20/15	0.41

*Chi square test

Table 2. ANOVA and post hoc LSD for the three groups

Baseline comparison						Multiple comparison# (p value)		
	Cinnamon group (a)	Chlorhexidine group (b)	Placebo group (c)	F value (ANOVA)	p value	(a-b)	(b-c)	(a-c)
Plaque index	2.4 ± 0.06	2.5 ± 0.06	2.5 ± 0.07	0.95	0.39	0.59	0.41	0.55
Gingival index	2.7 ± 0.08	2.9 ± 0.07	2.5 ± 0.08	0.89	0.41	0.33	0.36	0.21
15-day comparison								
Plaque index	1.9 ± 0.14	1.8 ± 0.24	2.7 ± 0.44	6.19	0.001*	0.609	0.001*	0.001*
Gingival index	2.0 ± 0.31	1.8 ± 0.40	2.7 ± 0.59	9.63	0.001*	0.748	0.001*	0.001*
30-day comparison								
Plaque index	1.2 ± .46	1.0 ± .48	2.9 ± .50	10.55	.001*	0.702	.001*	.001*
Gingival index	1.1 ± .57	0.7 ± .59	2.8 ± .55	15.71	.001*	0.817	.001*	.001*

Results are expressed as mean ± standard deviation. # Multiple comparison by post hoc LSD, * $p < .05$

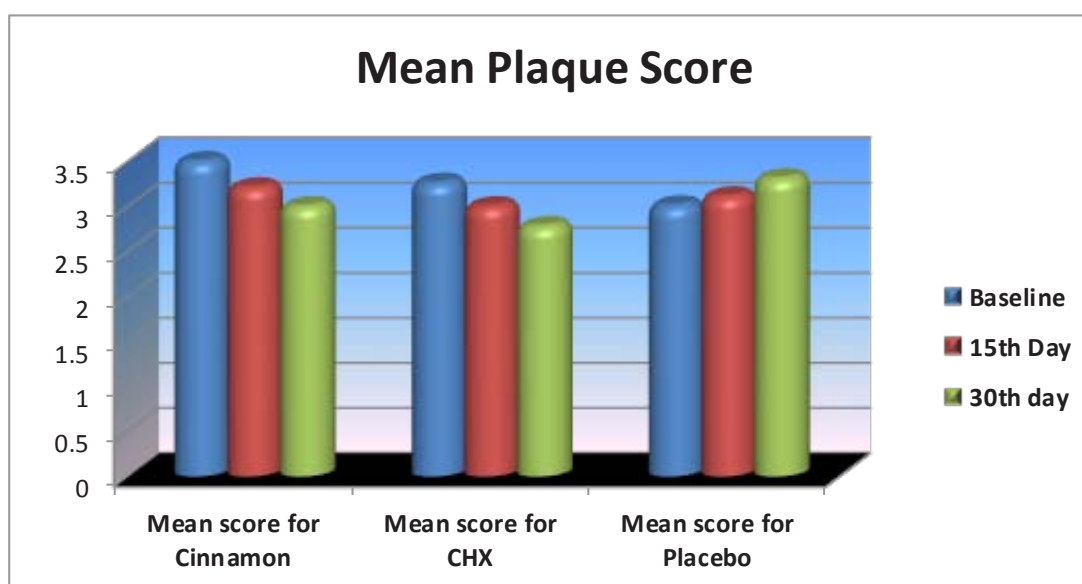


Figure 1: Mean plaque score for the cinnamon, chlorhexidine (CHX) and placebo groups at the indicated time intervals.

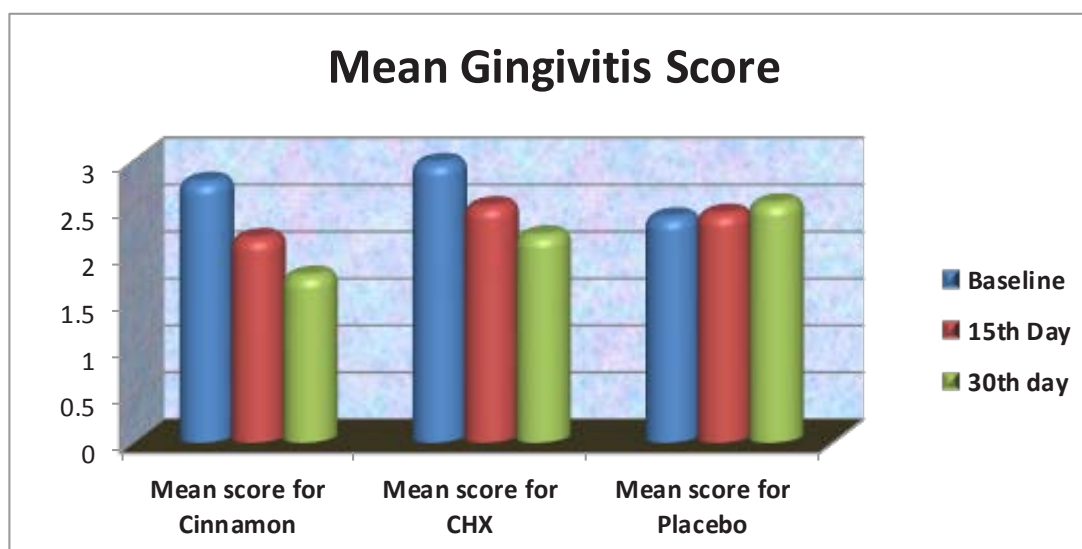


Figure 2: Mean gingivitis score for the cinnamon, chlorhexidine (CHX) and placebo groups at the indicated time intervals.

However, the differences between cinnamon and the placebo group, and chlorhexidine and the placebo group, were significant ($p < 0.05$). Data revealed no significant difference between cinnamon-based mouthrinse and chlorhexidine for any clinical parameters throughout the study. However, the placebo group showed an increase in plaque and gingivitis with the use of distilled water (Figures 1 and 2).

Discussion

The study was carried out to assess and compare the effectiveness of 20% cinnamon extract mouthwash, 0.2% chlorhexidine, and distilled water mouthrinse on dental plaque and gingival inflammation. There was a significant difference in the clinical level of dental plaque and gingivitis in both cinnamon and chlorhexidine mouthwash groups before and after the experimental period. Chlorhexidine showed greater reduction in dental plaque and gingivitis than cinnamon extract, but the results were not statistically significant. It can be said that both cinnamon and chlorhexidine were equally effective as anti-plaque and anti-gingivitis agents. The results of these two groups on plaque and gingivitis could not be compared with other studies, as no studies have been reported in literature that have tried to assess the same effect. We found a statistically significant difference between the use of chlorhexidine and distilled water, and between cinnamon extract mouthwash and distilled water.

In the present research, treatment doses were applied after breakfast and after lunch in order to eliminate the bias of decreased flow of saliva overnight, to which success in reducing the plaque index could be attributed. The students did not discontinue their routine oral hygiene practices. Students were instructed to rinse their mouth with 10 ml of solution for a period of 1 min after breakfast and lunch, and then were told not to rinse their mouth with water or drink anything for half an hour. A similar amount and duration of mouthwash administration was used in a study conducted by Gupta *et al.* (2014f). Chlorhexidine mouthwash used in our study did not contain alcohol. Todkar *et al.* (2012) stated that the alcohol-free rinse was as effective as one containing alcohol in reducing plaque and gingivitis.

In the present study, 20% cinnamon extract concentration was used based on a concentration analysis done by Aneja *et al.*, (2009). Aqueous extract was used because water is a polar solvent and dissolves almost all compounds in the plant. The antimicrobial activity of cinnamon on dental plaque and gingivitis, as observed in the present study, may be explained by its active substances: cinnamic aldehyde, an aromatic aldehyde and eugenol (Agaoglu *et al.*, 2007). Cinnamon bark is rich in cinnamaldehyde (50.5%), which is highly electronegative and interferes in biological processes involving electron transfer, and reacts with nitrogen-

containing components, e.g., proteins and nucleic acids, thereby inhibiting the growth of the microorganisms (Gupta *et al.*, 2008).

Removal of dental plaque is the mainstay in the prevention of periodontal diseases and maintenance of oral health. Periodontal diseases are claimed to be prevented by maintaining oral hygiene conditions that include reduction of plaque and prevention of gingivitis. Dental caries and periodontal disease are of great importance to deal with because of their high prevalence and their influence among individuals. Chemical and alternative preventive agents act as adjuncts to daily mechanical oral hygiene measures (Chang *et al.*, 2001; Biesbrock *et al.*, 2007; Schroeder and Ribeiro, 2004; Ximenez-Fyvie *et al.*, 2000; Radafshar *et al.*, 2010).

Chlorhexidine gluconate is, to date, the most thoroughly studied and the most effective anti-plaque and anti-gingivitis agent. The most commonly prescribed concentration is 0.2%; hence, this was considered in the present study (Brecx *et al.*, 1990; Lang and Brecx, 1986; Loe, 1976).

This study can be considered to comply with the requirements of an ideal mouthrinse protocol as specified by Barnett (2003). Additional inclusion of a positive control enhances the quality of the study (Gupta *et al.*, 2014; Gupta *et al.*, 2014g). Even though there are some promising results of the antibacterial properties of cinnamon, the clinical effectiveness of the anti-plaque and anti-gingivitis effects of cinnamon is not sufficiently defined at present and warrants investigation. So, the present study was designed to determine the anti-plaque and anti-gingivitis effect of cinnamon within the guidelines required by the American Dental Association Council on Scientific Affairs for evaluating the clinical efficacy of chemotherapeutic mouthrinses (American Dental Association Council on Scientific Affairs, 1997).

The *in vitro* study conducted by Fani and Kohanteb (2011), Gupta *et al.* (2011), Ayfer and Ozlem Turgay (2003) and Ohara *et al.* (2007) showed that cinnamon has strong inhibitory activity on *Streptococcus mutans*, which is the major causative bacteria of dental plaque. However, to date the efficacy of cinnamon extract has never been tested on the clinical level of dental plaque and gingivitis. To our knowledge this is first reported randomized controlled trial comparing the effectiveness of 20% cinnamon extract and 0.2% chlorhexidine on the clinical level of dental plaque and gingivitis, which limits the possibility of comparison with the literature.

The purity of cinnamaldehyde is more than 98% and it comprises 85% essential oil. Cinnamaldehyde has been proven to be active against many pathogenic bacteria (Ooi *et al.*, 2006; Suresh *et al.*, 1992; Wendakoon and Sakaguchi, 1995) including *S. aureus*, *E. coli* (Bowles *et al.*, 1995) and *Salmonella typhimurium* (Helander *et al.*, 1998).

It is among the most active component against Gram-positive and Gram-negative bacteria (Oussalah *et al.*, 2006). Cinnamaldehyde inhibits the growth of antibiotic-resistant and -sensitive *Helicobacter pylori* and hence prevents gastric ulcers (Campbell *et al.*, 2008). It has also proved useful against multidrug-resistant microbes causing nosocomial and community-acquired infections (Khan, 2009). The cinnamon bark also contains tannins consisting of polymeric 5,7,3',4'-tetrahydroxy flavan-3,4-diol units (Leung and Foster, 1996). Flavanols present in the bark might be responsible for the highly significant antifungal activity.

Most of the studies on cinnamon suggest that it is not harmful for humans and may be used as an agent to inhibit the growth of bacteria, fungi, and yeast (Gupta *et al.*, 2011). However, some cases of contact dermatitis and stomatitis associated with cinnamon oil have been reported (Gupta *et al.*, 2011; Khan *et al.*, 2009). Cinnamon is associated with allergic reactions with symptoms such as localized burning sensation, sloughing, erythema (Kumar and Sidhu, 2011; Siqueira *et al.*, 2009). Contrary to this, we did not find any cases of contact dermatitis, allergy or stomatitis in our study, which confirms its safe use as a mouthwash in the community.

Subjects of clinical trials might experience some improvement in oral hygiene not specifically associated with the properties of the test agent, but rather related to a behavioral change; this is known as the Hawthorne effect. Subjects enrolled in oral hygiene studies tend to improve their tooth brushing irrespective of the product they receive (de Oliveira *et al.*, 2008).

Though no specific test was employed for the substantivity, reduction in plaque and gingival score over a period of time were taken as criteria for the longer action of the mouthwashes. The results of the present study suggest that cinnamon extract has a plaque and gingivitis inhibitory effect which is almost equal to chlorhexidine mouthrinse. More studies with larger sample size on cinnamon mouthwashes should be encouraged to assess its efficacy, dosage, toxicity, formulas for patient recommendation, and long-term effectiveness.

Conclusion

Within the limitation of this trial, cinnamon has been shown to demonstrate similar effects on plaque and gingivitis compared to the bench mark control, chlorhexidine. The promotion of herbs with no side effects accompanied by low cost may motivate the patient at especially low socioeconomic strata for oral hygiene maintenance. This is an encouraging result which clearly favours promotion of cinnamon as a mouthrinse among rural communities, especially belonging to low socioeconomic strata, as cinnamon is easily accessible and is an inexpensive and safe alternative to chlorhexidine. However, as this is the first attempt to assess the effect

of cinnamon on plaque and gingivitis, clinical trials of longer duration with a larger sample size should play a vital role in commercialization of cinnamon extract mouthwash.

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